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A CRITICAL SURVEY OF THE PRESENT STATUS OF PLANT EMBRYOLOGY

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INTRODUCTION

At the outset of this article it might be stated that the most that can be said for the present status of plant embryology is that it is in a curious muddle. The main reason underlying this state of affairs is that very few botanists seem to realize that plant embryology has become an exact science. There are actually people who, in effect, deny that there is anything to this division of the botanical sciences. For instance, not long ago a botanist in India, while discussing certain species belonging to the Scrophulariaceae, made the following incredible statement: "A careful study of the development of the embryo shows that the stages are similar to those of a typical dicot embryo, and it is superfluous to describe these stages elaborately".

It is one of the commonest but most completely erroneous beliefs among botanists generally that there is such a thing as a "typical dicot embryo". Many authors dispose of the question of the embryogeny of the plants they investigate by stating that "it follows the normal Angiosperm (or dicotyledonous) type of development", blissfully ignoring the fact that no such development has ever been described. It is incomprehensible how such a "normal" method could be imagined to prevail for all angiosperms, or for either the dicotyledons or monocotyledons. This is but another example of the widespread misuse of the words "normal" and "typical" in plant morphology; sight seems to have been lost of the fact that what may be typical for one group of plants in some one respect is precisely as abnormal or atypical for other groups in the identical respect. We may recall that the so-called "normal type" of megagametogeny is common enough and may be "normal" for those plants in which it occurs, but it is quite abnormal for plants whose megagametogeny conforms to, say, the *Fritillaria* Type or

the *Oenothera* Type. Each of these types is in every sense "normal" for the plants in which it prevails; consequently, the only possible conclusion is that there is no such thing as a "normal type" for either megagametogeny or embryogeny.

One purpose of this article is to dispel the fatuous belief described above and to demonstrate, on the contrary, that it is far from being a superfluous matter to investigate the embryogeny of a particular plant carefully, cell by cell from the zygote to at least the later stages of proembryonic growth, and to publish such accounts, if only for the reason that the total number of angiospermic species which have been thoroughly studied embryologically amount to scarcely a hundred out of the many thousands of species which have been described.

The present account is concerned solely with angiosperms. Others are perhaps better qualified to discuss the gymnosperms; in any event, the embryology of this latter great group has been established on a more solid basis, thanks principally to the magnificent researches of Buchholz and his students, although the great mass of available information still remains to be compared and integrated as a whole. It should be mentioned in passing that this article is the outgrowth of the writer's activities during the preparation of a text on plant embryology (1); the opportunity is thus afforded to discuss a number of matters which could not be included in that book.

No distinction is being made between dicotyledons and monocotyledons, for the most part. It will perhaps be as much a surprise to many readers, as it was at first to the writer, to learn that there is absolutely no fundamental difference between these two taxonomic groups in so far as the ontogeny of the proembryo is concerned.

WHAT IS PLANT EMBRYOLOGY?

That would seem at first glance to be a stupid question, but as a matter of fact it is difficult to determine what many people understand by the term "embryology". Its meaning seems to vary according to the individual author. Many Scandinavian and German workers clearly apply it to embrace megasporogeny and megagametogeny alone with complete elimination of embryogeny. That is, they follow out all the phases from development of the ovule to maturation of the megagametophyte, and there stop. In a way,

since the series of events culminating in the formation of the egg are the precursors of the second series of events which end with the mature embryo, this might be described as embryology in an excessively broad sense. However, as a practical matter, it is confusing and deceptive; moreover, it is very annoying to the "pure" embryologist who is put to much trouble to procure obscure journals only to find that a title in which the word embryo is used in some connotation turns out to be misleading in that the embryogeny is not dealt with in any way whatever. There are literally hundreds of papers of this sort, all parading under false pretenses. Development of the ovule, strictly speaking, comes under the heading of anatomy. When megasporogenesis and megagametogenesis are separate processes, the first should be described as megasporogeny and the second as megagametogeny; when the two are consolidated into one process, it is simply megagametogeny. Embryogeny does not come into being until syngamy occurs; that phenomenon marks the commencement of the new sporophytic generation. Those who use the term embryology to cover all these processes overlook the fact that they are dealing with two separate generations, and with two aspects of one of these. Megasporogeny and megagametogeny in other than certain apomictic plants relate to the gametophytic (reduced or haploid) generation; embryogeny to the sporophytic (unreduced or diploid) generation, save where haploparthenogenesis is concerned. Again, development of the ovule concerns the preceding sporophytic generation.

All matters considered, it is far more preferable to consider embryology in the strict sense alone; that is to say, as it relates solely to the ontogeny of the embryo itself. Adherents of this conception may be described as belonging to the Souèges School; those who prefer the extremely broad conception described in the preceding paragraph might be designated as conforming to the Mauritzon School. To the Souèges School development of the embryo is the all-important matter; to the Mauritzon School, which has a decidedly sciolistic conception of embryogeny, development of the embryo as such is a relatively insignificant consideration. Each School doubtless has its place in the botanical scheme of things, but when one gets down to cases, it must be said that the Mauritzon School rarely makes an actual contribution to our knowledge of the precise embryogeny of the species which are discussed.

DEFINITION OF PLANT EMBRYOLOGY

As was indicated previously, plant embryology has advanced to the point where it is now one of the most exact of the botanical sciences. Few persons seem to realize, or to appreciate the significance of, this fact; the writer must confess that he certainly did not comprehend the extent to which our precise knowledge of embryology had grown until after a laborious digestion of Souèges' extensive writings. No one has hitherto called specific attention to the basic contributions of this French investigator. (A detailed discussion of Souèges and his contributions will follow.)

Plant embryology may be divided into two main divisions: (*a*) General Embryology and (*b*) Special and Comparative Embryology (1, 3).

The first division, General Embryology, may be subdivided into three groups of more or less related topics:

1. Embryonic Morphology. This embraces the static phases of embryology, and these in turn depend upon whether the external aspects alone are considered, in which case it may be described as Embryography, or whether specific internal aspects are under consideration, in which instance either Cytology, Histology or Anatomy is the term to be employed as the occasion requires.

2. Embryonic Physiology. This embraces the dynamic aspects of embryology. These aspects are very rarely dealt with by embryologists and are generally left to plant physiologists. One example is the cultivation *in vitro* of more or less immature embryos removed from seeds.

3. Embryogeny. These phases, with which the great majority of embryologists are concerned, embrace the kinematic aspects of embryology. They may be separated into individual phases, with appropriate designations, as follows:

a. Embryogenesis proper: origin of the embryo.

b. Embryotectonics: architecture of the embryo.

c. Embryogenergy: destination or functions of the embryo.

d. Embryonomy: exposition of the laws of embryonic development.

Special and Comparative Embryology require no subdivisions, since these topics relate to individual species, to well defined genera, occasionally even to entire families, and therefore follow phylogenetic lines more or less. There is a certain degree of over-

lapping, however, since species in very diverse and totally unrelated taxonomic families may conform to the identical embryonomy.

CONTRIBUTIONS OF SOUÈGES TO PLANT EMBRYOLOGY

One of the most inexplicable features of plant embryology of the present time is the extent to which the classical and fundamental researches of the French botanist, Souèges, are ignored. In fact, the general disregard of his work can be described only as enthusiastically unanimous.

Why this should be is difficult to comprehend. The writer has record of several papers on species which had been already fully investigated by Souèges, but in which no mention whatever of the earlier work is made.

A few biographical details regarding Souèges, since he appears to be comparatively unknown to American botanists, may not be amiss. Certain authors have given his initials as "M. R.," apparently in ignorance of the fact that the "M." is merely the abbreviation for Monsieur. His full name is Etienne Charles Réne Souèges. He was born in 1876. His academic training was not solely that of a botanist, as he has received French university certificates in mineralogy, biological chemistry, zoology and pharmacy as well as in botany; he acquired the doctorate from the University of Paris in the natural sciences in 1907. He has held no position corresponding to that of a professor in an American university; since 1912 he has been "Pharmacien en chef des Asiles de la Seine". The presumption is that his position is (or at least was, before the war) analogous to that of the head of a department of pharmacology in a medical school, but he, unlike so many American pharmacologists who make an avocation of the microscopic anatomy of the plant components of the materia medica, has made an avocation of plant embryology. In his later years, Souèges acquired a consuming ambition for election to the French Académie des Sciences, which had a certain deleterious effect on the mode of presentation of his investigations, particularly in the Comptes Rendus de l'Académie des Sciences. This ambition culminated in the appearance of his "Titres et Travaux Scientifiques" in 1934 (2); this, in brief, is an account of the personal scientific history and accomplishments which candidates for election to the Academy are required to present. The preparation and publication of such

a document may appear highly egoistical to an American; but that is merely the French way of doing things. The total number of titles of Souèges' published embryological papers exceeds a hundred; in addition, there are about ten brief books or pamphlets on such subjects as the history of plant embryology, the laws of embryogeny, and microtechnique as applied to embryology.

Souèges has placed descriptive angiospermic embryology on a high level of precision; it is so elevated, in fact, that only a remarkably few other workers, notably Bhaduri and Noll, have been able to comprehend, to criticize intelligently, and to duplicate with reasonably close imitation, his unique methods of descriptive embryology. Souèges has been a careful, painstaking and methodical worker; he has stated on several occasions that it required years of effort to work out the complete embryonomy of many of the species with which he dealt.

Souèges himself is not without his shortcomings. He is prone to direct little or no attention to the work of others in his brief articles which have appeared in the Comptes Rendus, although he does deal fully and completely with the literature in his more extended papers, particularly those which have appeared in the Bulletin de la Société Botanique de France. The first journal apparently places excessive emphasis on brevity. His descriptions of embryonomy in the Comptes Rendus are models of preciseness and completeness, but are not always strictly accurate from other standpoints. His use of the term "cellule apicale" for the outer cell of the two-celled proembryo should particularly be condemned; the writer might be in error in making this criticism if the term "apicale" has some meaning other than that of "apical" to a Frenchman. In any event, this cell, as has been recognized by several other workers, is not an apical cell in any sense of that term; it should be designated as the terminal cell. Souèges uses the term "dermatogen", in common with many another botanist, to apply indiscriminately to isolated epidermal initial cells, wherever these may be formed on the developing embryo, and has nowhere shown any indication of realizing that this word can apply only to the derivatives of a definite group of dermatogen initials located at the apex of the root proper, or from a group of dermatocalyptrogen initials. The epidermis of the embryo proper is never developed by such initials, but the epidermal initials arise independently in various portions, either

simultaneously or oftener at different developmental stages. The writer on occasion was unable to understand precisely what Souèges' conception of the root cortex included: at times he appeared to refer only to the cortex proper; in other instances he appeared to include all structures composing the partially differentiated embryonic root. This, obviously, is confusing. It would seem that Souèges is simultaneously at his best and his weakest when describing the origin and development of the primary root in the embryo. That is to say, he follows out the sequence of divisions in the radicular region with great precision, but is careless in his use of terms to describe the various component portions.

Despite all his personal idiosyncrasies, Souèges and his work on plant embryology can no longer be ignored by future workers in that field. Even if one fails despite strenuous effort to attain his level of clear description of embryonomic processes, the attempt should be made to do so. It should also be suggested that his mode of description of the various embryonal regions be retained; the few attempts that have been made to change his established zones of embryonal growth have resulted only in great confusion.

For the purpose of describing accurately the sequence of cell formation in the proembryo and embryo, Souèges has made it clear that these structures should be observed, described and figured with the terminal cell of the two-celled proembryo (or the embryo proper of the mature structure) pointed up or away from the observer, and with the basal cell (or the radicular end of the mature embryo) directed down or toward the observer. Numerous exponents of the Mauritzon School apparently never could make up their minds as to which way the embryo should be arranged; generally they depict it in exactly the same position that the mature megagametophyte is customarily figured—that is, with the micropylar end uppermost and the chalazal end lowest—or the reverse of the Souèges manner. Essentially, this matter should be one of no particular significance, since the megagametophyte and embryo in nature may both be oriented in any conceivable position, but it makes for greater stability and precision in description if the embryo be always considered from the one and the same position.

THE LAWS OF EMBRYOGENY

From a consideration of the great regularity present during the development of the proembryo of a given species, Souèges was led

to the formulation of a number of laws, each of which is truly specific when applied to that species. Those which succeed these fundamental characteristics during the ontogenesis of the embryo and which prevail in the development of definite tissues derived from the proembryonic initials or are already roughly delimited in the proembryo are not specific but are merely characteristic of the tissues in those individual species whose construction they govern. It is on this basis that the monocotyledons cannot be separated from the dicotyledons embryologically; during the second cell generation, the proembryo of the monocotyledonous *Lilium parryi,* to take one instance, divides precisely as does that of the dicotyledonous *Godetia amoena,* so that the two genera conform to exactly the same fundamental embryonomic type. It is not until much later that the secondary cotyledonary characters arise; such characters are not of basic importance.

The various laws may now be defined briefly.

(*a*). Law of Parsimony.—The pertinency of this law has apparently not occurred to Souèges, but it is most decidedly applicable to the field of plant embryology. In fact, it may be the fundamental law and all other laws may be derived from it.

As originally propounded by William of Occam (ca. 1270 to ca. 1350),[1] and known as "Occam's Razor", the law stated that "Entia non sunt multiplicandi praeter necessitatem". Later the same principle was discussed at greater length by Sir William Hamilton, a Scottish philosopher (1788–1856), and was called by him the "Law of Parsimony".

A very literal translation of the "Razor" is: "Entities are not to be multiplied beyond necessity". However, freer translations are possible and have been made. In so far as its application to plant embryology is concerned, this law may be stated thus: No more cells are produced by an embryo than are absolutely necessary.

The implications of this statement are obvious to any person who has ever carefully followed the development of the proembryo of almost any plant. Each and every cell has a reason for its existence; its origin can be demonstrated; its destination can be determined; and its position is invariably the same. A superfluous cell would seriously upset the harmonious balance of developmental

[1] The writer is indebted to Dr. Philip R. White for the information contained in this paragraph. See also: J. P. Givler, Occam's Razor and Mendel's peas. Jour. Elisha Mitchell Sci. Soc. **48**: 108–112. 1932.

factors present in the proembryo, and would undoubtedly lead to the death of the latter.

The one flaw in the rigid application of the law is that we do not yet know what is the real role or function of the suspensor, particularly in those Types or Variations in which the derivatives of the basal cell, which usually gives rise to the suspensor, contribute nothing to the construction of the embryo proper. In such instances the apparently superfluous cells of the suspensor may be disregarded.

(*b*). Law of Origin.—In any particular species, the sequences of cell formation may be established in such a manner that the origin of the cells may be defined in exact terms by referring to the one or to the other of the terms of the sequence.

It is hardly necessary to state that embryonic cells arise solely by simple successive bipartitionings of the zygote and its derivative cells.

The law of origin is the second most important of the embryogenic laws, and the principal branch of embryogeny, embryogenesis, rests on this law.

(*c*). Law of Numbers.—The number of cells produced by different cell generations varies with the species and depends upon the rapidity of segmentation in the cells of the same cell generation.

The rapidity of segmentation of a cell is calculated in determining the number of elements which the cell engenders in terms of successive cell generations. The law of numbers is identical with the law of rapidity and becomes evident in fundamentally different characters, as do the other embryogenic laws. Among other things it permits the establishment of particularly instructive resemblances between taxonomically diverse species.

The terminal cell of the two-celled proembryo generally segments more rapidly than does the basal cell and it usually gives rise to all of the embryo proper. The basal cell ordinarily contributes no more than the suspensor and portions of the root apex, and divisions occur less rapidly. There are instances, however, in which the basal cell segments more rapidly to form an extensive structure, and it may also contribute a greater portion of the embryo proper (the suspensor is considered not to be a portion of the embryo proper).

(*d*). Law of Disposition.—In the course of normal embryonic development, the cells are constituted by divisions in clearly deter-

mined directions and appear to occupy positions in accordance with the role which they must play in the future development of the embryo.

The laws thus far mentioned concern the relation of cells *to time,* while the law of disposition defines their relations *to space.*

All the dispositions which the cells of an embryo might assume are governed, in a fundamental manner, by the axial symmetry generally presented by both the proembryo and the embryo. This axis is identical with the axis of growth of the plant subsequent to germination.

The positional direction of the walls which segment a cell may be defined by taking account of their axial symmetry and by referring to the geometric figures which obey this symmetry. Cells separated by walls are thus similar to geometric solids: they possess the same faces, edges and angles, which allows the precise statement of their forms and the comprehension of their mode of assemblage. This branch of embryology which seeks to establish the laws governing the grouping of cells, the relations which they offer to each other in space, and which are applied to describe all the architectural dispositions of the embryonic body, constitutes embryotectonics.

(*e*). Law of Destination.—The cells of the proembryo of a given species, when development is normal, give rise to clearly determined parts, and always to the same parts, of the embryonic body.

This law appears to admit, in the fashion of a postulate, the specificity of the cell, besides which it affirms that each cell has a predetermined rôle to play. Among plants, the destinations of cells are not rigidly predetermined, since in addition to their actual potentialities the cells conserve a more or less considerable total of virtual potentialities which permits them to produce other parts of the embryonic body in case ordinary conditions of development become modified.

The law of destination is closely related to that of disposition. The first states, in effect, that the embryo and the plant represent a vertical edifice and that the fundamental regions of the body are situated in this edifice at different levels corresponding to the uni- or pluricellular embryonic stages. The law of disposition determines the mode of separation and the internal composition of the edifice.

The law of destination reveals the truly essential differences in embryogenic characters, in that it permits the establishment of the constant connections which, directly compared, constitute a source of precise indications of specific relationships between different plants. Determination of these relationships—in effect, the nature of the work performed by the embryo, the constructive rôle of its parts, and in sum the destination of each cell of the proembryo—is designated by the name of "embryogenergy".

CLASSIFICATION OF ANGIOSPERM EMBRYONIC TYPES

Up to the present time no one has attempted to formulate a definite scheme including all embryonomic types and variations so distinguished as to be readily recognizable. Souèges, in one or two of his later papers, mentions "Megaarchtypes", but the writer has been unable to find a scheme employing such terms in any of this investigator's publications that could be procured. Unsuccessful attempts were made to communicate with Souèges, both directly and *via* Swiss intermediaries, in an effort to ascertain whether he had actually prepared a classificatory scheme. Apparently he has done no more than to express the hope that a precise classification could some day be prepared.

The beginning, therefore, is the one made by Schnarf in his "Embryologie der Angiospermen" (4). He took the name of the family to which belonged the species whose embryonomy was most completely known, and designated it as the Type. It is clear that Schnarf was motivated by the fundamental laws laid down by Souèges, although he does not mention the fact, in his selection of the Types. Thus, he employed the terms "Cruciferen-Typus", "Astereen-Typus", Solanaceen-Typus", "Chenopodiaceen-Typus" and "Caryophyllaceen-Typus". Use of the designation "Type" appears to have become generally accepted, and the precedent established by Schnarf is fully worthy of perpetuation, since there is no acceptable alternative. However, the use of purely German names is open to objection; it seems to be better to take the root of the family name rather than the latter itself and to attach to this root the Latin suffix -ad. Schnarf's designations, with one exception, are retained in the revised form. His use of the Cruciferae (Brassicaceae) must be abandoned in favor of the Onagraceae, since species belonging to the latter family display a more funda-

mental type of embryonomy than does *Capsella bursa-pastoris,* the basis of the "Cruciferen-Typus".

The Types are based upon the operation of each and every one of the laws of embryogeny described above. Specifically, separation of one Type from another depends primarily on the plane of the first division in the zygote (whether longitudinal or transverse), secondarily, on the behavior of the terminal cell of the two-celled proembryo, when the plane of the first zygotic division is transverse, during the first and subsequent cell generations, and less directly upon whether the basal cell contributes something or nothing to the construction of the embryo proper.

The six Types which are now recognized may be best described briefly by means of a key:

I. The zygote divides by a purely or essentially longitudinal wall. Piperad Type

II. The zygote divides by a transverse wall.
  A. The terminal cell divides by a longitudinal wall during the second cell generation.
    1. The basal cell has no or only a minor part in the construction of the embryo proper ..... Onagrad Type
    2. Both basal and terminal cells contribute, more or less equally, to the construction of the embryo proper .....Asterad Type
  B. The terminal cell divides by a transverse wall during the second cell generation.
    1. The basal cell has no essential part in the construction of the embryo proper.
      a. The basal cell undergoes no further division and becomes a large suspensor cell (when a suspensor of two or more cells is formed, these are always derived from the terminal cell) ..... Caryophyllad Type
      b. The basal cell usually forms a suspensor of two or more cells ..... Solanad Type
    2. The basal cell contributes more or less to the construction of the embryo proper ..... Chenopodiad Type

No good purpose would be served by a fuller discussion of these types in the present connection, since they are described at considerable length elsewhere (1).

Under each Type there are from two to numerous subdivisions, styled Variations. Variations have never been specifically designated as such; they are being recognized for the first time in the writer's text. A Type is fundamental; a Variation is based upon modifications of the basic type. These modifications are more or less precocious differentiations, differences in the number of cells which participate in the construction of the suspensor, in the form or very variable dimensions of this structure, or even in its absence,

or differences in the rapidity of the segmentations which may occur earlier or later in favor of one of the cells produced by the same division. Variations are most conveniently designated by the name of the genus whose embryonomy departs in a minor fashion from that of the Type to which it is attached.

While it is a fairly safe assumption that the Types will stand the test of time, Variations are another matter. Not all are as clearly definable as could be desired. It was also recognized that as future research reveals the necessity, it is inevitable that new Variations will need to be erected under the appropriate Types.

The number of Variations is greatest under the Onagrad Type. Dicotyledonous genera so assigned include the following: *Capsella, Alyssum, Veronica, Catalpa, Myosurus* (including all Ranunculaceae), *Lythrum, Euphorbia, Mentha, Ruta, Trifolium* and *Lotus;* while the monocotyledons represented include *Anthericum, Lilium, Heloniopsis* and *Juncus.* Anyone familiar with the taxonomy of the angiosperms will recognize that these represent the most diverse families, yet all conform to fundamentally the same embryonomic Type and differ only in minor, secondary respects.

EMBRYOLOGICAL DISTINCTIONS BETWEEN DICOTYLEDONS AND MONOCOTYLEDONS

Some readers may question the statement previously made that there is no fundamental difference between the two great classical taxonomic groups among the angiosperms. The complete lack of recognizable basic differences was first enunciated by Souèges, whose reasoning leaves little or nothing for debate.

That there might be a difference arises from the fact that many authors have advanced the speculation that monocotyledony arose from dicotyledony through abortion of one cotyledon. This has never been proved as an indisputable fact, and can be shown by the embryogeny of almost any monocotyledonous plant to be palpably false. In effect, the two points of growth in the cotyledonary zone which occur among most dicotyledonous plants, in symmetrical position with reference to the embryonic axis, do not possess their homologues among monocotyledonous plants at any moment in the ontogeny of the embryo. The point of growth of the single cotyledon of monocotyledonous plants is located precisely on the embryonic axis, and no other symmetric point of growth exists.

Consequently, there can not be abortion of a second point of growth, since such a second point has never been found to exist at any time.

It is true, of course, that certain dicotyledonous plants have been described as aborting one cotyledon, thus attaining a pseudo-monocotyledonous condition. The one cotyledon which develops to maturity in such plants is always lateral with reference to the embryonic axis and is never truly terminal as in typical monocotyledons. Vestiges of the aborted cotyledon can usually be detected, generally at an early stage in the development of the embryo.

The embryological evidence, incidentally, would seem to furnish excellent arguments, if not conclusive ones, for those who favor complete abandonment of the separation of angiosperms into two groups.

APOMICTIC EMBRYOS

In the realm of apomictic phenomena conditions are extremely unsatisfactory. Various attempts have been made to straighten out the confusion, but from the embryological standpoint none of these endeavors has entirely succeeded in placing the subject on a sound basis. Some geneticists, in fact, have gone off on a wide tangent in an attempt to explain a purely embryological phenomenon on a basis of genetical terminology. It appears to be necessary to point out the fact that the mere presence of supernumerary megasporocytes, quartets, functioning megaspores or megagametophytes (whether normally or irregularly constructed) is in no sense proof that the plant concerned is regularly or sporadically apomictic. Such extra structures are far more prevalent than appears to be realized, and it is generally a matter of no particular significance. They are, for example, very common in *Fuchsia, Godetia, Lilium, Crepis, Nicotiana* and numerous other genera, but eventually disintegrate during later growth stages and never produce apomictic embryos. Indubitable proof of the presence of an embryo or two to several embryos in such supernumerary ovular structures must be presented before the plant in which they are found can definitely be considered as a regular or sporadic apomict. The majority of papers on apomictic phenomena have not presented such proof and consequently are of no value from the embryological standpoint.

POLYEMBRYONY

The situation with respect to polyembryony is equally unsatis-

factory. Buchholz has all but completely eradicated the confusion regarding it in the conifers, but no one has attempted to do the same for the angiosperms.

Matters depend considerably on the definition of polyembryony. Most authors apparently would define angiospermic polyembryony as the existence within the same ovule of two or more embryos, regardless of their origin. It is in precisely this respect that confusion lies. That is to say, no attempt is usually made to distinguish the actual origin of the various embryos that might occur. In most of the so-called instances of polyembryony, the supernumerary embryos are derived from the nucellus. The phenomenon has generally been styled "nucellar embryony", but the proper designation is "adventitious embryony" (to include instances of embryos arising from the inner integument), and such embryos must be classified as apomictic.

To cite another instance, if one of twin seedlings is haploid and the other diploid, it can ordinarily be taken for granted that the diploid seedling arose from a fertilized egg, while the haploid seedling developed either from an unfertilized synergid or from the nucellus or inner integument. Such an example should not be classified as polyembryony but as normal embryogeny accompanied by apogamety or adventitious embryogeny.

If we take the cue for the definition of true polyembryony from similar circumstances in conifers, there are only two causes of true polyembryony among angiosperms:

(*a*). A normally fertilized egg gives rise to a more or less multicellular body from which several potential embryos may arise. These extra embryos originate either from a massive suspensor produced by the basal cell of the two-celled proembryo, as in *Zauschneria latifolia* and certain orchids, from an embryonal mass produced mainly by the same cell, as in *Tulipa* and *Erythronium*, or by presumable budding from epidermal cells of the embryo proper, as in *Nicotiana rustica*. In most instances only one of the several embryos actually attains maturity.

(*b*). A filamentous proembryo first develops from the zygote, then its terminal cell splits longitudinally into two embryonal initials. Until very recently the evidence in all recorded cases was circumstantial, but the phenomenon has been authentically described in the orchid *Cymbidium bicolor*.

EXTENT OF EMBRYOLOGICAL STUDIES

A vast amount of careful work still remains to be done before the subject of plant embryology could be said to begin to show signs of exhaustion. The preponderant majority of angiospermic families have never been investigated with the slightest degree of completeness; in fact, there are many entire orders in which no species has ever been studied, save perhaps in a very superficial manner. These orders include the following: Anonales, Loasales, Podostemonales, Dilleniales, Coriariales, Pittosporales, Passiflorales, Theales, Tiliales, Malpighiales, Cunoniales, Garryales, Myricales, Balanopsidales, Casuarinales, Olacales, Meliales, Alstroemeriales, Dioscoreales, Agavales, Palmales, Cyclanthales and Haemodorales. Under still other orders a few families have been studied more or less, but the remaining families are still unknown embryologically. Under the Leguminosales, for instance, there are many investigations on both the Papilionaceae and Mimosaceae, but nothing whatever is known for the Caesalpiniaceae. Of course, it is recognized that many families, and even some orders, represent species which grow only in tropical or out-of-the-way localities; but nevertheless, many occur within easy access of properly equipped investigators in the North Temperate Zone.

THE HANSTEIN HISTOGEN CONCEPT

The original concept of the nature of histogens as devised by Hanstein, added to by Janczewski, and elaborated by Haberlandt, is plainly one that was presented far in advance of its time and thereby has suffered considerably. The bulk of the criticism has justly been directed against application of the theory to stem apices, far less to its application to root tips.

The only purpose of the writer in bringing up the subject is to point out one fact that seems to have been completely overlooked both by the originators and by later critics. It is fully conceded that the critics are on solid ground as far as their criticisms go; the point is that in no case has any one covered a particular phase completely. All writers have dealt with fully mature structures, whether they be stem apices or root tips, and in the case of the latter it has never been any too clear whether primary or adventitious roots were considered. No one seems to have begun with the actual origin of the histogens in the young embryo and worked

from that starting point up to maturation of the histogens in the seedling or adult plant.

It is the writer's strong contention that the start must be made in the young embryo—at the very first histogenic cell. Otherwise, only an isolated phase of the entire story is told. It is for this reason that it is believed that the histogen concept was prematurely presented; it was formulated at a time when nothing was understood of the laws of embryogeny which underlie the origin and development of histogens in a most fundamental manner.

It is impossible to comprehend how histogens arise, what their nature is, how the component cells are interrelated, and so on, unless the start is made in the embryo. The origin and development of the tissues of root tip and root cap can not be understood from a study of sections of adult tips. The same statement applies to the stem apex. Again, it needs to be borne in mind that the primary root has an origin entirely different from that of adventitious (lateral) roots. Structurally, the two may superficially appear to be identical, but that they actually can be so despite their totally different modes of origin has never been demonstrated.

The histogen concept needs to be restated anew from an entirely different standpoint, that of the embryonic origin of the histogens. It is entirely probable that the types of histogens found in a particular plant are intimately correlated, if not identical, with the Type and Variation to which that plant conforms embryonomically.

EMBRYOLOGY AND GENETICS

There undoubtedly exists a definite relationship between embryology and genetics. The latter term is here understood to refer mainly to crossing and hybridization experiments. Here the connection is clear-cut; in other aspects the interrelationships are still obscure.

A number of tentative embryological postulates relating to the crossing of closely or distantly related species can be laid down as a result of reflection on known embryological processes and known genetical phenomena. No attempt to prove these postulates is being made here; their presumable existence can only be suggested, and it remains for future investigations to demonstrate their validity or lack of such.

It is a well known genetical fact that zygotes resulting from

interspecific crosses divide once, then perish without growing beyond the two-celled proembryonic stage. What causes this arrested development? An investigation of the embryogeny of each of the parents should provide the clue. It is entirely possible that one parent may conform to some one set of embryonomic laws and the other to a different set of laws. Taking it for granted that conformation to a given set of embryonomic laws is an inherited character which does not permit of modifications in the same sense that differences in chromosome numbers can be reconciled, it follows that the hybrid zygote has received two conflicting sets of embryonomic characters in such instances where the respective parents conform to opposing embryonomic laws. Since the transverse division of the zygote is all but universal, the first division of this cell presents no difficulties, but these do arise when the time for the second division arrives. The incompatibilities between the inherited sets of embryonomic laws can not be reconciled, and death therefore ensues. To take a suppositious instance, let us assume that one parent conforms to, say, the Onagrad Type, in which the first division in the terminal cell of the two-celled proembryo is longitudinal, and the other to, say, the Caryophyllad Type, in which that division is transverse. What can the hybrid proembryo between two parents with such directly opposite characters do when the time for the terminal cell to divide comes? It can not divide in two opposite planes simultaneously. There is, in effect, nothing that it can do; therefore, it does nothing and because of the lack of further growth the proembryo perishes. It may therefore be postulated that the zygote of a hybrid between two parents with opposing embryonomic laws inevitably perishes. Furthermore, following the same line of reasoning, it may also be postulated that only those crosses are successful wherein both parents conform to the identical embryonomic laws.

In the preceding discussion, conformation to embryonomic Types was considered. There are numerous other recorded instances in which hybrid embryos developed into few- to several-celled monstrosities. These require an explanation somewhat different from the one given above. In such cases it may be presumed that each parent conformed to a different Variation under the same Type. Incompatibilities between Variations are not so difficult to reconcile as are those between Types, but it is still not always possible for the proembryo to overcome the differences completely. Ir-

regularities in construction of the embryo, generally manifested in the form of short-lived monstrosities, are all but inevitable in such cases. From this it may be postulated that in such instances where the parents of a hybrid conform to different Variations under a given Type, irregularities in the construction of the hybrid embryo are inevitable and generally end in the death of the latter.

It has now become quite clear that the basic reason why it is so easy to make interspecific and intergeneric crosses in the Onagraceae, despite other genetical or chromosomal differences between the parents, is that the entire family constitutes a remarkably homogeneous embryonomic unit. Differences in cytological or other genetical details clearly are not of as fundamental importance as is adherence to the identical cell-by-cell development of the proembryo and embryo. There are species which display divergences from the fundamental Type, particularly in the genera *Epilobium* and *Zauschneria*. It is demonstrable that when a form which either constantly or sporadically exhibits embryonal abnormalities is used as one parent in genetical experiments, the differences which this parent displays are reflected, usually in an exaggerated manner, in the progeny. At least there is no recorded instance in which the irregularities of one parent are overcome or erased in the offspring. All records reveal continued irregularities; the crosses frequently do not result in viable seed, and the cause, at least in those instances where sufficient details of the embryogeny were presented, is clearly due to abnormal development of the proembryo and its early demise.

It is apparent that future genetical investigations involving crossings between species and within the same species will be immeasurably enhanced in both theoretical and practical value if the embryogeny of both parents and offspring is taken into account along with cyto-genetical details. Those factors so far considered by geneticists in explaining their results are inadequate; the embryonomy needs to be evaluated along with the cytological and other genetical factors. It may, at least in very many cases, prove to be the deciding factor in determining the success or failure of hybridization experiments.

MICROTECHNICAL ASPECTS OF EMBRYOLOGY

It is admittedly a slow, tedious and tiresome job to prepare material of the majority of plants for embryological study. Not

many modern investigators seem to be possessed of the patience and perseverance of a Treub or Strasburger of the older school of botanists or of a Souèges or Buchholz of the present time to make thoroughgoing embryological investigations. Being an expert microtechnician is not alone sufficient; there is a good deal more to it. Strasburger, for instance, was one of the earliest microtechnicians, doing much of his sectioning with a hand razor, and his embedding and staining methods must have been crude in comparison with the modern procedures now available, yet his descriptions and drawings remain superior to many modern counterparts.

One needs plenty of patience and endurance to dissect out literally thousands of ovules which are tiny and difficult to manipulate in most plants, to get such quantities of material embedded, the skill to microtome the ovules in precisely the right plane to secure median longitudinal sections of the embryos, and proficiency in manipulating stains to show up the cell walls and nuclei to the best advantage for microscopic examination. Next there is needed the ability to interpret correctly what is observed under the microscope. One might make a drawing of every stage encountered, even repeating many times what appears to be the same stage, and particularly to watch for mitotic figures and to note carefully the planes of the spindles. These division figures are most important in determining the Type to which the plant under study might belong, since it is in this manner that one decides whether the terminal or the basal cell of the two-celled proembryo divides first and in which plane. The drawings may then be arranged in the proper sequence and each original cell and its derivatives may then be traced from one stage to the other and the latter may also be traced backward to their origin. The origin of the hypophysis initial or hypophysis cell, if either is present; the origin of the epiphysis, if it occurs; the contribution of the basal cell to the construction of the embryo proper, if any; the origin and development of the epidermal initials; the origin and growth of the hypocotyl; the origin and development of the root tip initials—all these and many other embryological features may be discerned by a careful study and comparison of the drawings by themselves and by comparison with published descriptions of the same stages in related species whenever such might be available. Determination of the Type is ordinarily based upon the first four proembryonic cell generations, but a complete series of

stages of the later phases of development are usually required for proper assignment to a Variation. Keys for the determination of the various Variations under each Type have been presented elsewhere (1); these keys, it should be noted, do not represent the limits of those which might eventually be recognized, so that one is at liberty to propose new Variations if these are so characterized that others may understand the features on which they are based.

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AIR-SPACE TISSUE IN PLANTS

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In an anatomical survey of the plant kingdom one is forcibly impressed by the widespread occurrence of intercellular spaces. They are particularly characteristic of parenchyma, and vary from the common narrow passages where cells seem to have pulled apart at their corners, to the irregular spaces of the spongy mesophyll of leaves, and the large chambers common in aquatic plants. These spaces usually contain a mixture of atmospheric gases. Their occurrence in the tissues of plants is so general that it constitutes the typical condition, at least in the higher groups, and they have reached a high stage of development even in some of the more massive Thallophyta. This situation, which contrasts rather sharply with that in the animal kingdom, opens interesting problems from the standpoints of development, distribution and function.

In view of these facts, it is not surprising that observations on air-containing tissues are widely dispersed through botanical literature. Morphologists, physiologists, ecologists and students of special plant groups have all been interested. The present object is to bring together the separate discoveries that have been made, so that their conjoint significance may properly be assessed. An inspection of the literature impresses the reader with the great trend toward specialization in modern science. The students of air-space tissue in non-vascular and in vascular plants have, almost without exception, kept their work in separate compartments, and, indeed, within these divisions a similar tendency toward isolation is observable when one reads, for example, the results obtained with algae and with Bryophyta. In attempting to bring the discoveries together, the lower plants will be dealt with first, and later the more advanced will be given consideration.

THALLOPHYTA

Fungi

The air spaces of algae and fungi have received little attention compared to that devoted to comparable structures in higher plants. They are by no means absent from these groups, however, and

detailed study, particularly in the fungi, might lead to interesting conclusions, for the lack of photosynthetic production of oxygen and elimination of carbon dioxide in the latter plants must make the air-space conditions very different from those in green plants. The hollow stems, with or without felted hyphae, characteristic of various massive fruiting bodies, and the spongy pseudoparenchyma of others, are used for identification, but I have seen little reference to their mode of development or their physiological significance.

In a few cases attention has been directed to the development of cavities in which asci or basidia are borne. In *Psalliota* (54) the primordium of the fruiting body is at first solid, but the hymenial primordium, which differentiates when the button is about a millimeter tall, soon pulls away from the tissues beneath, forming an annular cavity in which the gills with their basidial hyphae develop. In *Lycoperdon* (69) the hyphae in the interior of the young fruiting body pull apart in various places, forming cavities which contain very loosely woven hyphae with many broken ends. These cavities increase greatly in size as growth proceeds, and become lobed. The hyphae of the surrounding tissue grow toward the cavities, so that they appear bounded by palisade tissue. Basidia eventually develop from the hyphae of this palisade layer. It would appear that comparable developments occur in other Gasteromycetes and in those Ascomycetes that have perithecia. In fact, on looking through a modern text, such as that published by Smith (136), one is struck by the fact that in every case where asci or basidia are borne internally they are found projecting into rather large spaces previously formed. It would be interesting to know the composition of the gas contained in these spaces, and to investigate the mechanism by which ascogenous, basidial and other hyphae become oriented so as to grow into them. The fact that the asci or basidia occur only in such cavities or on free surfaces is also of interest. Even in a form like *Lophodermium,* a parasitic fungus whose asci are formed within the tissues of the host, an ascocarpic cavity develops as a preliminary to the production of asci (62).

Algae

In algae with massive bodies, intercellular spaces are commonly filled with the gelatinous substance of swollen cell walls. This

jelly is sometimes displaced by gas, however, even in forms not possessing special pneumatocysts. In this connection Oltmanns' (101) description of *Chorda* may be noted, in which he says the whole tissue mass encloses a central cavity filled with mucilage, or often with gases. Here and there diaphragms of proliferated hyphae cross the cavity. In *Enteromorpha intestinalis* also, accumulation of oxygen in the tubular plant body has been remarked (56).

The bladder-like floats that serve as supports for the fronds of numbers of the Phaeophyceae have become well known through the work of several investigators. They are plentiful among the Fucales and Laminariales, and are filled with gases.

In the Fucales rather careful morphological studies have been made of the bladders of *Fucus*. They develop near the growing point, in the medulla of the frond, and are said to be more numerous in plants of deeper water and in those growing in quiet places (111). First, a local thickening is observable, due to swelling of the gelatinous part of the cell walls. Then gas bubbles appear in the gelatinous matrix and enlarge, pushing the filaments of the medulla toward the outside (110). Richard (111) says the walls of these hyphae dissolve, a process not noted by the other investigators. The mature cyst is, at any rate, lined with a felted layer of filaments resembling the medullary hyphae (110, 166). Outside this are layers of ordinary cortical cells with their rather thick walls, and Richard described hyphae growing into the cavity from these cells. The outer cortical layers about the cyst are of thin-walled cells, gradually grading into typical cortical cells above and below, and the epidermis is normal and unmodified. It is emphasized (110, 111) that the gas in the bladders has no direct contact with the outer atmosphere.

Other members of the Fucales show minor differences. In *Ascophyllum nodosum* the main bladders are said to form early in May near the growing point, but smaller, secondary ones may form from time to time (80). The much divided, felted lining of *Fucus* is replaced in *Halidrys* and *Cystoseira* by a lining of parallel filaments, mostly unbranched (166). In *Halidrys* the bladders are divided into compartments by plates or cross walls of medullary parenchyma (110, 165, 26) with columns of long-stretched fibres uniting them (101), and *Halidrys dioica* displays every intergrade

between normal flattened fronds and well developed air bladders (27). Wille (166), who did not see the hyphae growing into the cavity of *Fucus* as later described by Richard, did describe short hairs extending into the space in *Ozothallia nodosa.* A similar production of hyphae was observed (27) in *Halidrys.* Such dissimilarities are important to the student of causal and experimental morphology, providing material with which he may work.

In the air-containing tissue of the Laminariales a series of forms with increasing specialization may be observed. Mention has already been made of the condition in *Chorda,* where gas may or may not have displaced the gelatinous matrix of the medulla.

In *Alaria fistulosa* the medulla of midrib and blade forms a series of distinct chambers (132), and their development is described as follows (66) : Long ragged slits appear along the median line of the pith. The tendency is to form one rift, rather than a number of small ones side by side. The rifts at this stage are in a vertical series, separated from each other by masses of hyphal chains with small clefts. The side walls of a large rift bulge outward slightly and are lined with torn hyphal chains. The ends are sharply angled, as if torn. The first true chamber, seen in slightly more mature tissue, has ends less sharply angled, and walls with fewer hyphal chains. The septa between chambers are more compact, and when they are mature there is no sharp line of demarcation between inner cortex and septum. The mature chamber has walls practically free from hyphae, and ends no longer acutely angled.

Miss Kibbe suggests that the explanation of this series of developmental stages lies in the strains produced by differential growth. The plant is supplied with the elongated, sieve-tube-like cells found frequently in brown algae. These cells, having finished their growth, become stretched, she believes, by the rapid division and growth of cells of the inner cortex. As a result of the consequent strain, the cortical cells are forced out of alignment, causing the walls of the midrib to bulge, and tearing the hypal strands that extend horizontally across the medulla. Longitudinal chains are eventually broken also, and between the breaks, *i.e.,* in the regions of the septa, the tension is somewhat relieved. Continued divisions of cortical cells enlarge the chambers and tear the pith, but the cells of the septa, where longitudinal strain is reduced, are able to withstand the lateral strain.

The hypothesis fails to explain the disappearance of medullary hyphae from the chambers. Even the fracture of the longitudinal ones requires more explanation if we are to assume the presence of inextensible sieve tubes that reduce longitudinal growth to a minimum. No experimental evidence was given to indicate great brittleness on the part of the hyphal walls, or especial toughness and inextensibility in the sieve tubes. Further examination of the development of these chambers would be interesting, in the light of more recent work on air-space tissue in higher plants.

It should be observed that Miss Kibbe is not alone among phycologists in suggesting such an origin for air spaces. A similar suggestion was put forward in 1885 with respect to the bladders of *Lessonia ovata* when the pushing apart of cells to produce the cavity was ascribed (46) to strong growth in area of the epidermal cells about the point concerned.

More specialized than the air-space tissue in *Alaria* are the gas-filled floats of *Macrocystis* and *Nereocystis*. The former (9) has a peculiarly developed, compound frond on which the pneumatocysts are borne in definite positions. Large primary fronds are produced, each with a thick cord running along one side of the lamina. Near the cord, perforations in the lamina occur which, by extension, cut the broad blade into a series of secondary fronds, each joined to the lateral cord by a short stipe. Of these the first few cut off (usually two) have no air bladders, but each of the succeeding ones develops one near its base. Two further observations on production of the bladders have since been made (95). One is that the primary frond may pass through a rest period and become rejuvenated, producing, as a result, more bladderless secondaries. The other is that a bladder may be formed in the growing region of a primary frond within the terminal blade, but when this has taken place further growth and cell division at the apex of that frond ceases. Where such bladders occur at all, they are usually fairly abundant and indicate general deterioration. It would thus seem that the production of a bladder, whether on a primary or a secondary frond, is associated with a limitation of growth in the tissues beyond it.

Development of the bladders was described (164) in 1885, and is not unlike that of the air tissue in *Alaria*. First, the intercellular jelly of the medulla swells in the locality where the bladder is to form. Then little air spaces appear here and there in the jelly. These

spaces grow and unite, forcing the loose medullary tissue toward the periphery, so that there is finally one large gas-filled space, crossed at intervals by individual hyphae. A later account (116) agrees with the above, except that the tissues of the pith are said to be gradually resorbed, so that in an old float this tissue has completely disappeared. A cambium produces secondary growth about the bladder.

The course of float development in *Nereocystis* is described as similar (81), there being at first rifts in the jelly, then a bubble increasing rapidly in size. The pneumatocyst wall has two cambia, an outer and an inner one. The inner is not found in other parts of the plant.

Physiologists have been interested in the pressure and constitution of the gas contained in algal pneumatocysts. The earliest analysis I have seen (114) gave pure nitrogen as the content of the bladders of *Fucus,* but this did not remain unchallenged. Wille (166), in bladders of Fucaceae under natural conditions in daytime, found 35–37% oxygen. After ten hours exposure to air the bladders contained only 20.7–20.8% oxygen, and ten hours in darkness reduced the proportion to 2.7%. A similar increase in oxygen content in daytime for the Fucales was obtained by Colla (20) who found this gas increasing in the forenoon and decreasing in afternoon. No carbon dioxide was identified by any of the above workers, and in this their results were in agreement with those obtained on gases from the vesicles of a number of Australian forms (78).

A group of American workers have published a series of reports on gas relationships in the floats of *Nereocystis.* The first of these (168) reports carbon dioxide varying from an average of 0.293% in daytime to 2.503% at night, while the corresponding percentages of oxygen were 12.304 and 10.906. Later papers, however, report that frequently the carbon dioxide is not present in appreciable quantities (70, 113), and the percentage of oxygen may be considerably higher. An interesting point was the discovery of carbon monoxide in the bladders (70, 113, 71). It was found in concentrations of from 1% to 12% (70). Its rate of formation was not influenced by light, but oxygen in the bladder was necessary for its production (71). Since it could not be produced by autolysis or fermentation of ground up plants (71), and since culture methods indicated bacterial sterility of the bladder cavities (112), it was concluded that the gas was a product of respiration of the plant itself.

The gas pressure in the bladders was found to vary from 124 mm. above atmospheric pressure to 316 mm. below. The pressure was higher in floating than in submerged bladders, and higher in frondless plants, and it decreased at night (41). Later, studies of composition and pressure changes in the gas of single specimens (113) led to the conclusion that the changes in pressure may be due to a change in oxygen content, with which they were always found to coincide.

BRYOPHYTA

Hepaticae

The conspicuous air chambers in the gametophytes of Marchantiales have attracted the attention of botanists from remote times. The members of the order have been classified into three types (36), based on the appearance of these chambers in sections of the mature thalli. In the *Riccia* type the chambers are in a single layer in the dorsal part of the thallus, and form rather long, more or less vertical canals, usually very narrow but sometimes broader. The *Marchantia* type also has its chambers clearly in a single layer, but they are broad, flat and polygonal, roofed by an epidermis, except for the central pore by which each communicates with the outer atmosphere. With few exceptions simple or branched photosynthetic filaments grow up from the floor of the chambers. On examining a section of the third or *Reboulia* type, one sees many irregular polyhedric chambers, in two or more layers, and often without but sometimes with cellular filaments or plates extending into them. That these chambers, whatever their arrangement, perform a useful function in placing extended areas of photosynthetic tissue in contact with air that is not excessively dry, is a thesis on which all can agree.

The course of development of the chambers presents points of interest and has given rise to some controversy. In this as in so many other problems of development, initial observations were made by Hofmeister. He held (59) that in *Marchantia polymorpha,* at the first appearance of the cavity, a single layer of cells is separated from the rest and lifted up by divisions in the cells that are to form walls between the cavities. He apparently did not observe the earliest stages, but clearly concluded that the origin of the chamber was endogenous and schizogenous. Kny (68) reported a schizogenous origin also for the canal-like cavities of the Ricciaceae but found the neighbouring rows of cells separating from without inward. Some

years later, however, in Leitgeb's comprehensive studies of the liverworts, he put forward a quite different concept of the origin and development of the air chambers in both Ricciaceae and Marchantiaceae (75, 76). He concluded that there was no separation of cells previously joined and no secondary lifting of the chamber roof from the underlying tissues, and his account of the origin of the chambers may be summarized as follows. At a point where four cells of the uppermost layer come together, the vertical growth of their lateral walls is less than in other places. This causes a little pit to form at the junction of the four cells. Next, a division parallel to the surface of the thallus occurs in each of the four cells, separating the part that forms the side of the pit from the rest of the cell. There is now a small pit surrounded by four cells, though no splitting apart has occurred. Successive horizontal divisions in these cells add to the side walls of the pit, which at the same time may be widened by extension and division of the cells in the thallus beneath. The roof which covers the broad chambers of forms like *Marchantia* has its beginning in oblique divisions of the top cells of the side walls. This description had the advantage, in Leitgeb's view, of bringing the air chambers into harmony with the depressions that contain sex organs. These organs, when very young, project above the thallus, but they later become overgrown by the surrounding tissues.

For more than 25 years workers on liverworts seem to have been in unanimous agreement with Leitgeb (42, 15, 16, 17, 45, 13), but in 1907 Barnes and Land (3) joined issue with him. Describing in detail the growth of chambers in a number of genera, they found them originating invariably by the splitting apart of cell walls within the thallus. The great majority of later writers agree that, in the forms they studied, the chambers have originated by splitting, beginning either from the outside (25, 100, 104, 53, 102, 103,) or from inside (107, 143, 30, 52, 102, 133, 48) the tissues. Perhaps an indication of the cause of some of the divergence of opinion as to the place where the splitting starts may be gathered from Evans' (36) work on *Grimaldia fragrans,* in which he found that the splitting was sometimes from within, sometimes from without. Two workers (58, 7) since 1907 have reported forms in the Ricciaceae developing as described by Leitgeb. Commenting on the latter paper, Evans holds that the drawings are not convincing and that evidence

against splitting from without was not presented. In the former, four species are described as differing from the description of Barnes and Land. Of these, three were studied only from soaked herbarium specimens, and the drawing of the fourth suggests splitting. Campbell, however, in a later edition of his text on mosses and ferns cites these papers and that of O'Keefe (100) as substantiating Leitgeb's hypothesis.

Once the air chamber has been initiated, it grows by active enlargement and division on the part of the surrounding cells. On this all are agreed and no one has found any evidence of enlargement by the death and disappearance of cells, as is seen in the lysigenous spaces of higher plants. The pronounced tendency toward cell growth and division about the young air chambers is so general in these forms as to merit special mention. In many species it is indicated not merely by the increase in size of chambers but by the growth of filaments (144, 15, 3, 25, 87, 52, 102, *etc.*) or plates (96, 36, 30, 104) of chlorophyll-bearing cells into them. A number of writers have reported these plates as forming complete partitions across the chambers in the forms they studied (16, 13, 124, 133).

There seems little occasion for doubt that chambers of the *Riccia* and those of the *Marchantia* group are fundamentally similar, their differences being explainable on the basis of different growth reactions on the part of the surrounding cells after the chambers have been initiated. In fact, some species of the *Riccia* group have chambers not unlike small ones of the *Marchantia* type. Juel (64) describes in *Riccia Bischoffii,* in the region of the midrib, chambers in the form of narrow canals, each bounded by four rows of cells. Out in the wings, however, the canals become progressively wider as the distance from the midrib increases, till the broader ones become spaces surrounded by 6 to 15 cells. He states that the broader canals on the flanks arise from the greater radial growth in that region.

Evans (36) names 12 genera among American liverworts alone, with chambers in two or more layers. The deeper ones have been supposed by some (16, 124, 133) to be parts cut off from the primary chambers by the plates growing in from floor and walls, a plate in such a case growing across the chamber and fusing with the wall on the other side. Others disagree with this. It seems clear (3, 30, 53, 36, 102) that the secondary chambers connect up with the

primary dorsal ones, and the tendency to think of them as merely extensions of these, produced by extension of the splitting to deeper cells of the thallus (30, 53, 102), would, if correct, place the air spaces in liverworts more in line with those in higher plants.

On the development of air-space tissue in liverworts other than *Marchantiales,* little has been written. In Jungermanniales the assimilatory tissue is spread out in thin plates, and air chambers are lacking. Of the Anthocerotales in general it is said (3) that triangular and quadrangular spaces occur in both sporophyte and gametophyte, similar to those typical of the parenchyma of vascular plants. In addition, many members of this group have large lacunae in their gametophytes. Of the 291 species described in Stephani's *Species Hepaticarum* (145), 130 are described by the term *frons cavernosa* or *costa cavernosa,* indicating the presence of such lacunae.

From the standpoint of causal morphology, the air chambers of liverworts have been accorded just sufficient attention to indicate that further experimental work might produce results of considerable interest. The genus *Dumortiera* is interesting in that typical air chambers are normally more or less completely eliminated from the mature thallus. In *D. hirsuta,* Coker (19) noted that individuals growing on a gentle slope where spring water kept them continually wet, had no sign of chambers. Those growing on moist sand in a cave, however, produced near the growing point well formed chambers with irregular pores and scattered papillae. The chambers disappeared as the tissues became more mature, but the drier conditions in the cave clearly favoured their development. In *D. velutina* and *D. tricocephala,* Ernst (35) reported the upper epidermis completely lacking. The chambers were consequently roofless, but their side walls developed and papillae projected from their floors. In *D. trichocephala,* even the side walls and papillae were finally eliminated from older parts of the plant. This writer also noted that the amount of reduction was influenced by water relations. Campbell (14), in *D. trichocephala* from Hawaii, found no rudiments or vestiges of air chambers. In other species of the genus, chambers were evident near the tip, and the side walls might persist, though the upper epidermis always disappeared. Insofar as the action of water is concerned, Campbell found in *D. calcicola,* which grew in dry places, well developed

chambers near the tip, and side walls and papillae remaining in older parts. He emphasized that moisture was not the only controlling factor, however, as two species growing together and under identical conditions with respect to moisture had different degrees of suppression of the chambers. Experiments with *Dumortiera* might suggest the mechanism through which moisture acts on this very sensitive tissue, and thus add to our knowledge of cell ecology.

Two researches on *Marchantia* and its air chambers give ground for hope that here also the experimental morphologist might find material for investigating the effect of water on these structures. As long ago as 1893 it was observed (117) that in submerged plants there were in many places no chambers, and where they did develop they were shallower than normal and contained a less luxuriant growth of assimilatory filaments. Only a few of the chambers were filled with air. The report of the inhibiting effect of submergence in water on air-chamber production was confirmed in the latter of the two papers(40), but this author was inclined to think that the limiting effect of the presence of water was an indirect one. He noted that the spaces that did contain air were normal, though the whole plant was submerged in water during their development. The air chambers of *Fegatella* are also affected by submergence (28), though perhaps to a lesser extent.

Cutting down the light intensity has been found to have an inhibitory effect in three genera investigated (5, 28, 39). Various colours of light were tried on *Marchantia* (39), and while in red light the chambers were normal, in blue they contained no assimilatory filaments, and in green almost none was produced. This is interesting in the light of Teodoresco's finding that chambers and filaments were very luxuriantly produced in concentrations of 1.5–2% carbon dioxide and were much reduced when thalli grew in the absence of this gas. Perhaps their development is bound up with photosynthesis.

Musci

The moss gametophyte seems to be entirely lacking in schizogenous spaces such as we have described for the liverworts. There is no suggestion that the air-enclosing outgrowths on the upper side of *Polytrichum* leaves are anything but outgrowths. We have here, however, active growth and division of chlorenchyma cells in contact with air, entirely comparable to that concerned in

the enlargement of liverwort air chambers, and in at least one species of moss (160) this growth was found to be inhibited by darkness, as it was in some liverworts. Moss gametophytes in general, like those of Jungermanniales, are characterized by the arrangement of their green tissue in thin plates, a form of body not conducive to air-space development.

The stem of the moss gametophyte is a column many cells thick, and the central part of this sometimes contains cavities. Such spaces were found by Lorch (77) to be normal in at least two species, of different genera. In *Campylopus polytrichoides,* he was able to state that the cavity was lysigenous in nature, produced by the resorption of the central strand of the stem. Remains of the dead cells were still observable in the cavity at maturity.

The sporophytes of mosses are commonly green when young and exhibit a many-layered assimilatory tissue comparable with that of the vascular sporophytes of higher plants. In this tissue, air spaces among the cells are present in most if not all species (72, 49, 50, 158, 159, 61, 77, 162, 163). The small interconnecting spaces described by Haberlandt (49) in the chlorophyll-bearing tissue of some forms, opening to the outer air through the stomata, were found often to extend to the apophysis and, even, in some cases, to the seta (158, 159, 61, 163). Vaizey (158) found such spaces present also in the seta of *Atrichum undulatum,* a form without stomata. Lorch (77) speaks of spaces in the seta as characteristic of the families Polytricaceae, Dawsoniaceae and Buxbaumiaceae. He looks on these as capillary conductors of water, however, and not as air spaces.

In special parts of capsule and apophysis it is very common for these spaces to enlarge greatly by rapid growth and division of adjacent cells, and in this way are produced the large lacunae so common in the chlorophyll-bearing tissues of moss capsules (72, 49, 159, 61, 77, 76, 162, 163). Often the separation, growth and division of cells to produce these lacunae proceeds with almost diagrammatic regularity. It has been described in detail for *Polytrichum Juniperinum* (162).

Lysigenous spaces may sometimes be present in the seta. Vaizey (158) observes that a large space of this category is found in the centre of the leptoxylem in *Splachnum sphaericum,* and while Lorch (77) classified the spaces he found as schizogenous, van der

Wijk (163) states that they become larger through collapse of cells, which observation, if correct, would place them in the other category.

VASCULAR PLANTS

In the sporophytes of vascular plants, air spaces are so common that there is a tendency to take them for granted. A survey of the literature fails to find a plant in this group recorded as without them.

Small Air Spaces of Unspecialized Tissues

The small intercellular spaces that are the forerunners of all air spaces in primary tissues, appear very close to the apical growing point. For details of development in this region we are indebted to the work of Schüepp (129) and Priestley (108). The rapidly dividing cells at the growing point have no intercellular spaces, being plastic in nature and closely pressed together. Very soon, however, differentiation begins, and a change from rapidly dividing, non-vacuolated cells to more slowly dividing, vacuolating cells is accompanied by the appearance of small spaces, as if the cell walls had become elastic and the enlarging cells had rounded up and pulled apart at the corners. At first these spaces are filled with liquid, but very soon the liquid is replaced by air, and at the same time, according to Priestley's work, the change from a slowly vacuolating, dividing cell to a rapidly extending cell takes place. The small air spaces have by this time united to form a continuous system which, in the aerial shoot, connects with the outer air through the stomata. In many tissues the aerating system ceases development at this stage, but not infrequently larger spaces develop in various ways from this beginning.

Before passing on to consider the larger spaces it is necessary to discuss a problem in connection with these first stages of development, a problem that presents itself when the constitution of the cell wall and the composition of the lining layer of the air spaces is investigated.

Meristematic cells are separated from one another by a middle lamella of a pectic nature, and inside this pectic envelope each protoplast has completely surrounded itself with a thin primary wall of a combination of cellulose and "pectin" (156). The word "pectin" has often been rather loosely used in the literature to indicate soft pectic substances. Microchemical evidence indicates the presence

of pectic acid rather than true pectin in young growing points (23, 88, 135), but in the present connection the difference is not important.

In the decade from 1880 to 1890 there was some controversy as to the lining of intercellular spaces. Earlier contentions that the substance was protoplasm (118) have been overcome, and present workers are in agreement with those (122, 167, 83) who held that, except in special cases where some lignification or suberization may have taken place, the lining is composed of the pectic substance of the middle lamella. The original mistake was perhaps due to the fact that near the growing point microchemical reagents used to test for cell wall substances usually fail to react characteristically, a fact held by Priestley's school to be due to the presence of fats and protein in the walls in this region (156).

The spaces that form at the corners of cells might, in harmony with the above, be held to be due simply to the splitting apart of the middle lamella at these points, but when one considers in detail the formation of the new wall at cell division the insufficiency of this simple explanation becomes evident. The meristematic cell about to divide is completely surrounded by two layers, the middle lamella and the primary wall. After cell division a middle lamella is laid down in the cell plate separating the two daughter cells, and a layer of primary wall forms on each side of it. The new middle lamella joins up at its edges with the side walls of the parent cell, but between it and the old middle lamella of the parent cell is the thickness of that cell's primary wall. It is at such a corner that a small intercellular space forms when the cells begin to vacuolate, and such a space when mature is always completely lined with middle lamella substance in continuity with the middle lamellae of adjacent cells. There is no evidence of a break in the pectic layer, and a problem arises as to what becomes of the primary wall layer that originally separates the newly formed middle lamella from that of the parent cell.

Workers in Martens' laboratory (65, 84, 85) undertook a series of developmental studies with attention directed to this point, and in primary and secondary tissues of a number of forms they reported the following. When a cell divides, the new middle lamella is separated from the old one about the original cell by a layer of cellulose wall. The free ends of the new middle lamella then

spread or thicken so that in transverse section a triangular mass is seen. This mass enlarges and becomes a cavity, triangular in section, surrounded by pectic substance of the middle lamella. As the cavity grows, the cellulose wall between it and the middle lamella of the parent cell breaks or is dissolved, and the cavity thus becomes an intercellular space. Sometimes the older middle lamella splits opposite the expanding end of the younger, and in such cases the two spaces fuse when the cellulose has been broken down. Confirmatory evidence has been obtained (109) from macerations of collenchyma, sclerenchyma and the parenchyma of young internodes.

Larger Air Spaces in Primary Tissues

Although many tissues are characterized by the above type of air space at maturity, it is a common occurrence for development to proceed further, culminating in larger spaces than those described and often in the large lacunae characteristic of water plants. Viewed morphologically, plants and tissues that have attained more advanced types of air spaces appear to belong to two separate groups, and while much still remains to be done in causal and experimental research, it seems, in the present state of our knowledge, that this classification depends on rather pronounced differences in cell metabolism.

The least accentuated example in the first group is perhaps to be found in the spongy mesophyll tissue characteristic of large numbers of leaves. In this tissue the spaces described above continue to increase in size. Instead of the turgid cells pushing their rounded contours into the sharply angled spaces, it comes about that the walls are drawn in at those points where they are not in contact with other cells, and the spaces between them become rounded out. In more pronounced cases the tissue is finally composed of extremely irregular cells maintaining contact with their neighbours by means of arms projecting in various directions, so that the tissue becomes a loose air-containing meshwork. In some leaves this tendency extends also to the palisade cells (94, 150, 157), whose sides are finally connected only by projecting arms.

It has been observed (150) that in leaves with both palisade and spongy parenchyma, cell division ceases earlier in the cells of the spongy tissue, and the intercellular space system depends on the ex-

tent of the "stretching stage", which, in turn, is initiated during the development of the stomata. This fits in well with Priestley's observation that the period of rapid cell expansion in stem tips begins with the entrance of air into the then minute spaces.

Satisfying experiments with leaves to show the mechanics of the production of the spongy parenchyma cells are lacking, but some researches on the relative volume of air spaces in different leaves and under various conditions are of interest to students of causal morphology. In measuring the size of the air spaces the majority of workers have used methods based on that originated by Unger (157) in 1854. He estimated their volume by comparing the weight of pieces of tissue in their normal condition with that attained when the spaces had been injected with water by the use of an air pump. Two refinements of this method may be mentioned. Kisser (67) pointed out that the use of the air pump sometimes results, unless extreme care is taken, in the harming of delicate tissues, and recommended the use of a centrifuge as a subsidiary method; Nius (98) used turpentine instead of water for the infiltration. Another method, in which the areas and perimeters of spaces as seen in microscopic sections are measured, has also been used (106) but less frequently. An idea of the proportion of air by volume in normal leaves may be obtained from Unger's results. Among the 41 species he investigated, he found a minimum proportion of 77 parts per 1,000 in *Camphora officinalis*, and a maximum of 713 parts per 1,000 in *Pistia texensis*. Later investigators have found that conditions have a significant effect on the volume of the spaces. Leaves grown in shade have a larger proportion of spongy parenchyma (141) and a larger volume of air spaces (142, 147, 128). The air-space volume seems to vary with the water supply (98, 128), but to a much lesser degree in some plants than in others (98). Evergreen leaves of the temperate zones were found to have a smaller air volume than deciduous (98), and in soft evergreen leaves this volume decreased in autumn (128). In a number of leaves it has been found that spaces develop well in white or blue light, poorly in red, and very poorly in green or in complete darkness (149).

We may now proceed from the spongy tissue of a normal green leaf to consider the more extreme modifications exhibited by such forms as *Typha* and *Juncus*. The young *Typha* leaf has in the

beginning a homogeneous parenchyma (38), the cells of which grow and pull apart much as has been described, but the process proceeds further than in most leaves. The final result (92, 139) is that in the leaf-sheath there are large lacunae filled with a very loose tissue of long-branched cells, very thin-walled, and mostly free from the lacunar walls, while in the blade are seen typical lysigenous lacunae from which the loose tissue has disappeared. Here and there in both sheath and blade are places where the metamorphosis of cells has not gone so far and diaphragms are left. Those in the sheath are composed of cells similar to the loose ones in the lacunae but with their vertical arms short and their horizontal ones remaining attached to the sides. In the blade the diaphragms are composed of short-armed cells, thick-walled in the basal diaphragms but thin-walled near the leaf tip. A somewhat similar situation is reported in *Juncus inflexus* (32) which may have on the same root, stems with continuous pith, with pith interrupted at certain regions, with pith almost entirely disappeared, or with pith partially disintegrated leaving a ring of canals. In Pandanaceae (139), as in Typhaceae, some of the spaces contain branched cell tissue and some do not. Such stellate cells are also to be seen in adult spaces in *Scirpus* (137) and could doubtless be found in other forms.

Large lysigenous air spaces are common in water plants and are found in numbers of land plants as well (60, 127, 57, 88, 165). Some attention has been paid to the causal aspect of their development. As long ago as 1855 Leitgeb (74) was interested in this aspect and his paper includes an outline of literature back to Malpighi and Grew. He thought the cells became star-shaped by secreting air into the small spaces and enlarging them while adhering to each other at the points where their walls were in contact. Duval-Jouve (31, 34) came next with the idea that the cells were pulled and stretched by continued growth of the surrounding tissue, and in this way the arms were drawn out at points where the walls of adjacent cells adhered. He said that true lacunae were formed because the stretched cells could not keep pace with the growth of the surrounding layer, and their torn remains could be seen adhering to the sides of the mature canal. The same conclusions seem to have been arrived at independently by some later workers (146, 134), and Newcombe (97), after an experimental

study with 24 different plants, concluded that the tension of tissues is a factor in limiting the life-period of the cells which normally collapse in cavity formation. He found, however, that although the development of a cavity could be deferred by artificially preventing extension of the surrounding tissue, the cells that normally collapsed would finally die without this extension.

The hypothesis of the shaping of the armed or stellate cells and their final rupture and death by the pull of a growing ring of surrounding tissue does not fit with the fact that often lysigenous spaces are surrounded only by layers of soft tissues that would be easily drawn inward and could not produce the required tension. McPherson (88), from his experiments on cortical spaces in roots, arrived at another hypothesis which is more in accord with the facts as they are known at present. In the roots of *Zea Mays* the series of morphological changes leading to the formation of an air space was the usual one. First, the cortical cells passed through the normal period of rapid vacuolation with small spaces at the corners, and this period, with the cells turgid, pressing against each other and rounding out into the spaces, endured throughout the time the root was increasing in diameter. Later, the cells in the region where an air space was to form, began to collapse. Where the walls of adjacent cells touched, they adhered, and thus the collapse of the cell gave it the peculiar, irregular shape, with projecting arms. There was here no question of the arms being drawn out by growth of the ring of surrounding tissue, for such growth had previously ceased. Later the protoplasm of these cells became progressively thinner and finally disappeared, the soft cell walls collapsed completely, and a lacuna resulted.

The hypothesis was that the active protoplasm of these cells used up food materials more rapidly than they were being supplied by the conducting system, and then destroyed itself by respiring its own proteins, as is commonly done by starving cells. It was found that immersion in water, or any other procedure that brought about a lowering of the oxygen supply, produced larger lacunae, a finding in agreement with those of numerous workers (21, 22, 99, 8, 29, 12) and one to be expected according to McPherson's hypothesis, if one remembers the especially rapid breakdown of food material in anaerobic respiration. In fact, the concentration of oxygen about the root could be increased to the point where no lacunae would be

produced. In experiments with a number of other plants it was shown that each had its own critical oxygen pressure, below which lacunae would form.

One more condition was essential for the normal formation of lacunae. They developed normally when the cell walls were composed of cellulose and pectic acid, as revealed by microchemical tests, but not in those where the pectic constituent was calcium pectate. In the latter case cells died and their protoplasm disappeared, but the hardened cell walls retained their normal shape.

Later a similar origin was suggested for the lysigenous spaces in the leaves of *Ledum groenlandicum* (135). In this case the cells reach an abnormal size before collapsing, actually pushing out the soft lower surface of the leaf into little mounds. Some evidence is produced that this rapid increase is due to an increase in permeability to water on the part of the protoplasm. Such permeability increase would continue in protoplasm undergoing autolysis, until complete permeability replaced semipermeability and collapse resulted.

This brings up to date the account of investigations of lysigenous spaces. Whether the early stages of their development are fundamentally the same as those producing normal spongy mesophyll may some day be determined by physiological experiment. The morphological similarity is very close.

With regard to the development of schizogenous lacunae, one feels a need of further investigation. Even a casual inspection reveals the profound difference between the reaction of the cells concerned in their production and that of those forming the lysigenous spaces. In both cases the beginning is the same, small air-filled chinks developing among the cells, but beyond that the similarity ends. The cells that are to form the schizogenous lacunar tissue act as if stimulated by contact with the air, growing and dividing rapidly. Thus the original small spaces, triangular or quadrangular in cross section, become large smooth-walled cavities or canals, walled by the progeny of the three or four cells or tiers of cells that originally enclosed them.

In some cases the cell divisions producing the lacunae follow a course of extreme regularity. Sauvageau (120, 121) has recorded this for the leaves of *Zostera*. In this plant the very young mesophyll is of cells elongated transversely of the leaf. These

draw apart at the corners, leaving small spaces. At the same time a very small cell is cut off from one end of each elongated cell, adjacent to the space. Both elongated cells and small ones now undergo a series of divisions. The daughters of the elongated cells form the sides of the lacunae, while those of the small ones form the diaphragms, one cell thick with small and usually triangular pores. He listed seven other genera of aquatic monocotyledons with similar development, on one of which (*Enhalus*) his findings have been confirmed by later work (24). Others had spaces of less regular growth.

The pronounced tendency toward growth and division on the part of cells touching spaces in plants that form schizogenous lacunae is well illustrated in the peculiar diaphragms of *Nuphar,* the development of which has been observed repeatedly (153, 47, 11). The air canals are at first uninterrupted by cross diaphragms, but certain cells of the walls form swellings which project into the cavity, branch and divide rapidly, forming masses of loosely arranged cells across the canals.

In one case the development of large schizogenous spaces unconnected with increased cell division has been suggested. Hallquist (51), in the pneumatophores of *Lobelia exaltata,* described large cortical lacunae, apparently schizogenous, into the production of which cell division seemed not to have entered. A study of the development of these lacunae would be of interest. They are described as having single layers of radially stretched cells between them. In *Myriophyllum* an identical arrangement of tissues has been described (161) as arising by tangential divisions of the cells separating the spaces, followed by radial stretching.

Before passing from the consideration of lacunae in primary tissues, something more should be said of the diaphragms by which, in many forms, they are interrupted at intervals. These diaphragms attracted the attention of earlier anatomists who recorded their presence in numerous genera and families (154, 18, 152, 33, 38, 125, 119, 44). When Le Blanc (73) stated, as an indication that their existence does not depend entirely on aquatic environment, that they are found only in Monocotyledons and the related Nymphaeaceae, he had apparently overlooked one or two papers. The great majority of plants in which they have been found do belong to these groups, but they have also been reported in *Utricularia* (152) and the Haloragidaceae (44).

The diaphragms assume a number of forms. In *Nuphar* (153, 154, 47, 11) and *Brasenia* leaves (125) we have the air canals occluded by loosely-packed masses of cells, outgrowths from the canal walls. In most plants with diaphragms, however, they have a definite tissue form. The diaphragms are most commonly one cell thick, but may have a thickness of two or three (34, 91) or several (33, 74) cells. In a number of cases they are traversed by veins (32, 33, 34, 73), and one writer (73) has suggested the support of these veins as one of their functions.

In most plants the diaphragms are perforated by large numbers of very fine pores. Sometimes the pores occur only at the corners where more than two cells were originally in contact, in which case they are presumably produced like those among the star-shaped cells of developing lysigenous spaces, the main difference being that the arms on the diaphragm cells usually remain quite short. Often, however, the cells draw apart, not only at the corners but at many other places, so that each cell appears in face view surrounded by numbers of short finger-like processes whose tips are in contact with the tips of similar ones of adjacent cells. This enlarges greatly the number of minute pores in the diaphragms, and incidentally raises an additional problem of development. We need to explain the pulling apart of cells at numerous places along their sides, while they closely adhere at intervening points. Goebel (44) suggested that the cells adhere by the thickenings of their walls, while remaining free to separate at the pits, a hypothesis not very popular among later writers who, however, have not succeeded in solving the problem.

The question of the contribution of environmental conditions to the development of diaphragm cells has also proved difficult. According to Le Blanc's account (73), a swelling and rounding out of young diaphragm cells gave rise to small angular spaces at their corners. This was followed by a shrinkage of cell bulk, perhaps due to evaporation into the spaces, and this shrinkage, together with a stretching of the whole diaphragm by growth of the surrounding tissues, accounted, in his opinion, for the final size and shape of the spaces. Snow (137) suggested that in *Scirpus validus* the formation of a diaphragm resulted from a retention of meristematic properties on the part of the cells of a layer, while those on either side ceased growth and became the slender stellate air-space cells.

She found (138) that a decreased rate of growth was associated with an increase in distance between diaphragms. In general, such a decreased growth rate followed a change from water to air or from high to low temperature. There were many exceptions, however, and satisfying results on the causal morphology of the diaphragms are still lacking.

Air Space Tissue from Phellogen

Cells of the phelloderm layers produced centripetally by cork cambium behave much as parenchyma cells, drawing apart at their corners to produce the characteristic, small, angular spaces. The cork cells, on the other hand, remain tabular in shape, their walls becoming suberized before they swell and round out by vacuolation, and so they usually lack intercellular spaces.

The complementary cell tissue of lenticels provides an illustration of special air-space tissue produced by phellogen. It is stated (26) that lenticels are found in all great groups of vascular plants and on all their organs, but generally only in those plants and organs with secondary growth. Trécul (155) observed their origin in 56 species and found them developing under stomata in all. They are not always limited to this position, however, and have been classified on the basis of their origin (26) into two groups, namely primary ones, developing early on a spot determined by an organ such as a stoma, root or bud, and secondary ones developing late at other points.

The development of a lenticel was followed and described more than seventy years ago (140). In *Sambucus nigra* the following stages were noted. Under a stoma in a twig, cortical parenchyma was found to extend to the epidermis, displacing the sub-epidermal collenchyma. The parenchyma cells of this group enlarged and divided, along with some of the collenchyma cells, so that the epidermis was stretched and a small lump appeared. The cells rounded up and thus produced intercellular spaces and became a mass of complementary cells. Inner layers became a cambium, producing complementary cells toward the outside and phelloderm toward the inside, and the epidermis was torn by this increased growth. In some other forms the same author notes that the phellogen is responsible for even the first steps in lenticel formation, and it is only after a layer of cork has been laid down that swollen complementary cells are produced.

In the mature lenticel, layers of complementary cells alternate with layers of cork, each cork layer sealing the lenticel until broken by expansion of later formed complementary cells. The complementary cells constitute the air-space tissue. Devaux (26) has noted that in some lenticels even the suberized cells of the closing layer round up, presumably before suberization, and produce spaces.

It seems undeniable that the production of complementary cells instead of cork cells is bound up in some way with abundant water supply. When a corky layer has closed the lenticel, the inner tissues are protected from water loss, and complementary cells are formed. When a twig is placed in water or in air saturated with water vapour, it is normal for so-called water lenticels to form with no closing layers but a continuous production of complementary cells. Devaux (26), who reported this, found it quite possible, by regulating transpiration, to change a water lenticel to an air lenticel and *vice versa.* On the other hand, it has been found that in the roots of cotton plants the lenticels became much more hypertrophied under boiled water than under tap water periodically aerated (148). If this reaction is general, it would seem that water, in addition to its direct effect, brings about a secondary stimulation of complementary cell production by cutting down the oxygen supply.

In a number of plants this tendency to produce unsuberized cells and air-space tissue under the influence of an aquatic environment extends to the whole of the phellogen. Of such plants at least 39 species have been investigated (86, 115, 105, 43, 63, 130, 123, 126, 6, 10, 131, 89), and numbers of additional ones could doubtless be found.

The cells of aerenchyma tissue so produced have been found to have no suberin in their walls (123), and often possess the further specialization that they assume definite forms and so produce a regular tissue structure in place of the irregular mass of cells and spaces in the air-containing portion of a lenticel. A relatively unspecialized type may be seen in *Sesbania* and *Neptunia* (115, 130, 89). The tissue here is irregular. The only advance over the tissue of complementary cells in a lenticel is seen in a tendency to elongation and sometimes branching on the part of the cells, which thus produce a rather more porous mass. The more ad-

vanced type, as seen in *Nesea verticillata*, has been well described (126). The cells cut off from the phellogen are flat and oblong, but soon round off so that their tangential faces are in contact only over a small central part of each cell. Then certain individual cells in each tangential layer push out bulges in a radial direction. The result is that successive layers are pushed apart. The intervening spaces become filled with air and are interrupted only by the beam-like projections from the special cells, which hold the layers apart.

Submergence in water provides the stimulus for the production of aerenchyma. Plants that normally produce it were found, when growing in dry situations, to have none (123), but to produce cork instead, and in *Nesea* (126), branches that bend over and dip in the water form aerenchyma instead of cork on the submerged parts. Seliber (131) has, however, pointed out from his work on *Jussieua* that good nourishment and some aeration are also requisite. Wholly submerged plants failed to produce aerenchyma. Moreover, the aerenchyma grew better in good light and the amount of light necessary for good growth varied with the species. A thorough understanding of the problem awaits experiments with all these factors definitely controlled.

Gaseous Content of the Air Spaces

A survey of investigations of the gas contained in the tissues of vascular plants serves to emphasize three points, namely, the ubiquity of gas-containing tissue, the high proportion of oxygen in green tissue when exposed to light, and the positive correlation between carbon dioxide content of spaces and vital activity, in tissues not engaged in photosynthesis.

Even from succulent plants or from fleshy or woody organs, gas for analysis can be obtained, and the results of analyses are instructive. In the air from inside sugar beets (55) the oxygen content varied between 0.06% and 2.10% and the carbon dioxide between 11.49% and 78.90%. In five species of succulents (2) analyses of gas taken on a sunny day showed oxygen content of 18.35% to 26.45% and carbon dioxide content of 0.41% to 1.50%. In apples, potatoes and carrots in storage (82) the percentage of carbon dioxide in the intercellular atmosphere rose and that of oxygen lowered significantly with increases in temperature, but

always the former was more plentiful and the latter less so than in normal air. In gas extracted from the trunks of trees (79), the carbon dioxide varied from 0% to 26% and the oxygen from 0% to 22%. In branches and roots of grape vine and mulberry (37) the oxygen was found to decrease and the carbon dioxide to increase with increasing vegetative activity. When inactive the percentage of carbon dioxide was very low, and the oxygen content approached that of the air.

In an earlier section of this paper it was noted that gas pressure in the floats of *Nereocystis* increased at times to points very considerably above atmospheric pressure and that this increase was contemporaneous with an increase in oxygen content. The same has been found to be true in the intercellular spaces of aquatic angiosperms, and gas from their lacunae will, in daytime, when oxygen is being set free by photosynthesis, be forced out in bubbles which can easily be collected. Van Tieghem (151) found such gas, bubbling from wounds in *Elodea canadensis,* to be 90% oxygen.

GENERAL CONSIDERATIONS

The distribution of air-space tissue in the plant kingdom is interesting. While one group of water plants, the algae, has less of this tissue among its massive forms than any other, in a second group, the aquatic angiosperms, air spaces reach their greatest development, both in individual size and in combined comparative volume.

One cause of the scarcity of spaces in algae is undoubtedly the character of the cell wall, particularly its primary layers, whose tendency to swell with water into a gelatinous mass must impede or often indeed preclude the development of the small schizogenous spaces so characteristic of higher plants. This also would militate against the development of large spaces of the schizogenous type if the suggestion we have made should hold, namely, that such spaces arise from special growth of the cells bordering the original small ones.

Such wall peculiarity fails to account, however, for the scarcity of lysigenous spaces amongst the algae. This, it seems to me, may be accounted for by a physiological pecularity of these primitively aquatic plants. Diffusion of gases through water is very slow as compared to that in air, and, as shown in an earlier section of this paper, there is reason to believe that this, through its effect on res-

piration, causes enlargement of the lysigenous cavities in many higher plants. In the algae, however, we have to deal not with a group that has evolved for ages in air, with continuous ready access to oxygen, but with one whose cells have always been immersed and have a constitution that enables them to function normally under such conditions.

In the few cases where algae do produce spaces there are differences between them and the higher plants, and also similarities. In vascular plants, at first small, angular, liquid-filled spaces form from the pulling apart of cells. Normally they are soon united with each other and with the outside, and become filled with air. Whether this air comes from outside, or is secreted by surrounding cells, or owes its origin to both factors, there is still insufficient evidence to decide, though the first would seem unlikely in the case of submerged water plants. On the other hand, in vascular plants, the early shape of the spaces is not such as one would expect, if gases had been secreted into them under pressure. Moreover, in many Marchantiales, where the schizogenous spaces are reported to be in continuity with the outer atmosphere and full of gas from the first, the probability seems to be in favour of the entrance of outer air. In seaweeds, the air spaces, when present, do not connect with the outside. Careful studies of the earliest stages of their development seem rarely to have been made, but in *Fucus,* where such stages have been observed, they are said to appear as bubbles in the gelatinous walls, and it seems reasonable to assume that the gas responsible for their initiation and growth comes from the cells. The picture here, as obtained from evidence obviously still incomplete, is that of cells giving off gases into the interior of the frond and thus producing the bladder. In the Laminariales, even if the theory of Miss Kibbe with respect to *Alaria* should prove to hold for the group, and the spaces be found to be due to the tearing effect of differential growth, the gas with which they are filled can come only from the surrounding tissue. Miss Kibbe's hypothesis can not at present be accepted without reservation, however, for its explanation of some of the facts is not completely satisfying.

The large spaces which almost invariably develop in seaweeds where a beginning has been made as described above, may be compared to the large lacunae of the higher plants. In *Alaria* and *Lessonia* enlargement of the cavities was held by some to be due to

stretching and tearing of the tissues by rapid growth of the surrounding cells. In higher plants the same origin has been suggested for the lysigenous spaces, but subsequent work has thrown doubt on this theory, and a hypothesis of autolysis has been suggested. In so far as schizogenous lacunae are concerned, developmental study has been scarce except in the Marchantiales, where the original impetus arose from Leitgeb's view, considered erroneous by most modern workers, that no schizogeny occurred. In these chambers development is clearly bound up with a tendency to copious cell division on the part of cells in contact with the moist air of the space, and what we have seen of the form and development of schizogenous lacunae in vascular plants is indicative of the same tendency.

Some kinship between the lacunae in seaweeds and those in higher plants may be seen in two particulars. Three workers have reported in three genera, the disappearance or resorption of cells during the development of spaces, and in four, a stimulation of growth has been indicated, either by the growth of hairs or hyphae into the cavity, or by the development of an active cambium around it. It would thus seem that the fundamentals for the production of both lysigenous and schizogenous lacunae are present in some degree in the algae.

In Bryophyta the schizogenous type of air space predominates, though lysigenous spaces are found, particularly in parts of mosses not well supplied with chlorophyll.

The description by Martens and his school, of the first beginning of an air space, not by a mere splitting apart of cells but by inflation of the end of a newly-formed middle lamella after cell division, is interesting and seems reasonable in the light of our knowledge of young cells. If the chinks at the corners between cells come as a result of a rounding up process which in turn is due, as Priestley holds, to vacuolation and to elasticity of the primary wall, the break must come at the point where coherence of the intercellular substance is weakest, and it is to be expected that this spot will be found in the soft watery lamella that has just been laid down. We know that the middle lamella retains much of its softness for some time, and the fact that splits sometimes occur in the older side lamella in this same region of strain fits in very well with that fact. The occurrences at comparable points in the history of cell walls of Bryophyta and Thallophyta have yet to be investigated, and the problem may

prove difficult, for Martens found it possible to get a clear picture of what was taking place only in favourable material.

Whether the irregularly armed cells of the spongy mesophyll of leaves are physiologically akin to the star-shaped cells found in some mature lacunae and produced as an ephemeral stage in the development of others, is a question that still awaits a definite answer. If they are, something must be assumed to happen in normal leaves to halt their progress along the course they have begun, a course that, followed to its conclusion, brings about their destruction. That happening might be the attainment of a more copious supply of oxygen or of food. Both would be available in greater quantity in leaves when photosynthesis reached normal proportions. Obviously other possible factors might be mentioned, but in the present highly theoretical stage of the problem, additional theorizing would not be profitable.

Before closing this paper some observations are in order on the place occupied by air spaces and lacunae in the plant's economy. Two possible spheres of usefulness present themselves as worthy of consideration, namely, aeration and, in the case of water plants, buoyancy and support.

In algae the aerating rôle of air-space tissue is of little consequence. In the bladders of *Nereocystis* it has been established (113) that oxygen accumulates in daytime when photosynthesis is active and disappears during the night, doubtless being used in respiration by the surrounding tissues. There is nothing to indicate, however, that these tissues are the better, either for this stored oxygen or for the supply of respiration products that accumulates during the night and disappears during photosynthesis. The tissue in the vicinity of the bladder has not been found to fare better than the much greater mass of cells constituting the rest of the plant or than the same tissue in youthful fronds without bladders. The ancestrally aquatic thallophytes are constitutionally in accord with an environment where diffusion is slow, and the additional retardation provided by the more or less swollen and gelatinous matrix of cell wall through which diffusion to and from the inner cells is free to take place, seems ordinarily to be harmless. The bladders do, however, serve as floats and supports for the massive, flexible, plant bodies.

Just as algae may be said to have their inner cells in direct communication with the water outside through the water imbibed in the

cell walls, so are the inner cells of land plants in contact with the outside air through the interconnecting intercellular spaces. Thus cells whose normal reactions require more rapid diffusion than is found in a liquid medium are able to function deep in the structure of the plant. The contention that this free and rapid transfer is important to such cells is upheld by the fact of their death in some cases when it is interfered with, as in McPherson's experiments, and also by the increased growth and cell division about the air spaces in plants that develop lacunae schizogenously.

Photosynthetic tissue in land plants, whether liverworts, mosses or vascular plants, is ordinarily provided with a particularly impressive system of air spaces, and the value of a free road for the diffusion of gases to and from the cells of such tissue will scarcely be denied.

Workers with aquatic angiosperms have given thought to the question of the benefit, if any, that these plants derive from the large lacunae so plentiful in their tissues. To the present writer it seems that the situation here is very different from that in seaweeds, and that these lacunae may be held to perform a useful function in providing for transfer of a supply of oxygen from photosynthetic cells to others. Diffusion of oxygen through water is much less rapid than that of carbon dioxide, a fact that has been held (1) to account for the increase of pressure in lacunae during photosynthesis. It has also been shown to be a factor in the death of cells of land plants under water, and remembering that aquatic angiosperms are descended from land plants, it is reasonable to assume that, since they are successful in their new habitat, this hazard must in some way have been eliminated. It is quite clear that, during the day, the concentration of oxygen in the lacunae of green plant parts becomes abnormally high. If this oxygen is to be transferred in any quantity to the lacunae of buried parts where, if anywhere, a special need for it would exist, the lacunae must form a continuous system. Barthélemy (4) gave evidence that this is so in *Nelumbium* and *Nymphaea*. He found not only that by blowing air into a cut petiole he could force it out through the stomata of the leaf blade, but also that by placing one leaf of an intact plant under reduced pressure in a belljar full of water, he could cause a continuous stream of air to enter the stomata of the free leaves, pass down through the tissues of the plant, and emerge into the belljar through

the stomata of the leaf enclosed therein. This experiment could probably be performed successfully with numbers of other water plants. It was done quite independently with *Sagittaria* some years ago, by an undergraduate in an ecology laboratory class at Toronto.

With a continuous system of lacunae from leaf to root, the diffusion of oxygen from the point of high concentration in the leaf toward the place of scarcity in the tissues without chlorophyll would seem inevitable. In addition there is the possibility of a certain amount of mass movement, the carbon dioxide of respiration from these tissues becoming dissolved and diffusing away, thus allowing room for an influx of oxygen-rich air from the upper lacunae where it is present under pressure. In plants with their leaves above water, a similar transfer of oxygen from the outer air through the lacunae might be expected during darkness. Something similar is also probable in the aerenchyma tissue whose development from cork cambium has been described, for this tissue extends to the surface of the water and often if not always to a point a little distance above.

It remains only to mention the importance of the buoyancy of the air-space tissue which largely compensates for the scarcity of mechanical tissue in submerged and floating angiosperms.

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